THE CLASTOGENIC EFFECT OF TARTRAZINE, A SYNTHETIC YELLOW DYE, IN PLANT MERISTEMATIC TISSUES

BONCIU ELENA[1*], ROSCULETE ELENA[1], ROSCULETE CATALIN AURELIAN[1]

[1]University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania

*Corresponding author email: elena.agro@gmail.com

Keywords: food colorant, clastogenic, Allium, consumers

ABSTRACT

Food dyes is used both in commercial food production and in domestic cooking. One of the colorants is tartrazine (Tz), a synthetic lemon yellow dye. The purpose of this paper was to highlight the clastogenic effect of Tz to plants meristematic tissues, using the *Allium* assay. Three different concentrations (0.3, 1 and 1.3%) were used, the exposure time being 6 hours. The statistical analysis of the obtained results indicates that with the increase Tz concentration, mitotic activity is inhibited, while the chromosomal aberration rate in the cells in mitosis as well as the frequency of nuclear abnormalities in the interphase cells increases. The main genetic abnormalities identified were laggards, stickiness, C-mitosis and micronucleus. These results suggest prudence regarding the consumption of processed foods which containing tartrazine.

INTRODUCTION

Either the synthetic food colours or natural food colours, the colour has always had an important implication on the minds of people as far as food is concerned. It is therefore necessary either to preserve the natural or maintain the characteristic colour of a food product while it is manufactured or stored for future use (Bonciu, 2017; Bozhanska, 2018; Righi et al., 2018; Georgieva et al., 2018).

According to FDA, a food colorant is “any dye, pigment or substance which when added or applied to a food, drug or cosmetic, or to the human body, is capable (alone or through reactions with other substances) of imparting colour” (FDA, 2016).

Tartrazine is a synthetic yellow azo dye that is used as a food coloring. The E number of tartrazine yellow is E102. Other name of tartrazine is FD&C Yellow 5. It is popularly used in drugs, particularly in shells of medicinal capsules, syrups, cosmetics, fruit cordials, coloured fizzy drinks, instant puddings, cake mixes, custard powder, soups, sauces, ice creams, ice lollies, sweets, chewing gum, marzipan, jam, jelly, marmalade, etc. Tartrazine yellow is also used in many convenience foods along with glycerine and honey products.

Some people may be intolerant to Tartrazine. Although previously banned in Norway, Austria and Germany, E102 has been deemed safe for use by the European Food Safety Authority which has recommended a safe level of consumption. The UK Food Standards Agency called for a voluntary phase-out of E102 by 2009. In the EU, food and drink products containing E102 must carry the label warning ‘may have an adverse effect on activity and attention in children’ (https://www.safefood).

We considered this cytogenetic study to be appropriate for evaluating the cytogenetic effects of tartrazine (Tz) by using the *Allium* assay. *A. cepa* has assayed to be best model plant for standard use in environmental monitoring and cytological analysis (Bonciu, 2012; Bonciu, 2018; Bonciu et al., 2018).

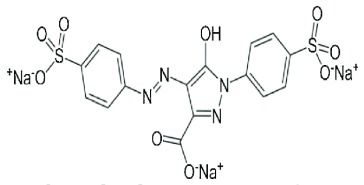

Fig. 1. Chemical structure of tartrazine

MATERIAL AND METHOD

The biological material used was represented by small sized onion bulbs, which were immersed in glasses with water for 72 hours, time required for the meristematic roots occurrence. When the meristematic roots reached the length of 1.5 cm, they were immersed in dilutions of various concentrations of the Tz (0.3, 1.0 and 1.3%) for 6 hours, at room temperature. A number of 10 onion bulbs were used for each treatment variant as well as an untreated control that was immersed in tap water.

The roots were processed according to the protocol of fixation, hydrolysis and staining with Schiff reagent (Feulgen method).

The microscopic slides were performed according to the squash method. Statistical analysis was done using MS Excel 2007. The analysis of variance (ANOVA) was used to assess the significant differences between the control variant and each treatment. The differences between treatment means were compared using the LSD-test at a probability level of 0.05% subsequent to ANOVA analysis.

The mitotic index was calculated using the following formula:

$$MI\ (\%) = \frac{\text{Total number of cells in division}}{\text{Total number of analysed cells}} \times 100$$

The index of the total abnormalities (TA) was also calculated:

$$TA\ (\%) = \frac{\text{Total number of aberrant cells}}{\text{Total number of cells in division}} \times 100$$

RESULTS AND DISCUSSIONS

The results are showed in Table 1. The cytotoxicity level can be determined by the decreased rate of mitotic index. It was found that Tz induced a strong clastogenic effect in meristematic cells of onion. The clastogenic effect was enhanced as the concentration of Tz increased. Thus, compared to the Control variant, the mitotic index recorded a decrease from 28.12% (Control) to 20.22% (V2), 11.28% (V3) and 7.55% (V4).

The decrease in the mitotic index was positively correlated with increasing concentration of Tz solutions.

Several studies have been oriented to demonstrate the clastogenic effects of some food additives and pointed out their danger as carcinogens or mutagens. Some authors reported that the mitotic index of *A. cepa* root tips was successively decreased with the increase in different dye concentrations and duration of treatments (Vazhangat P. and Thoppil J.E., 2016).

In our investigation, all treatments with Tz resulted in a significant increase of TA (Figure 2). The main genetic abnormalities identified were laggards; stickiness; C-mitosis and micronucleus. TA index recorded an increase in all treated variants, from 1.53% (Control) to 13.65% (V2), 24.46% (V3) and 30.09% (V4). The increase in the TA index was positively correlated with increasing concentration of Tz solutions.

Laggards' chromosomes and C-mitosis were the dominant abnormality induced after treatment, especially at higher concentrations. In Figure 3 are shown some cytogenetic abnormalities identified in meristematic roots of *A. cepa* exposed to different concentrations of Tz. Cytogenetic abnormalities occur under biotic and abiotic stress conditions (Bonea, 2016a; Bonea, 2016b; Bostan et al., 2013; Butnariu, 2012; Ianculov et al., 2005; Samfira et al., 2013). This can affect the selection of parents in plant breeding program to develop new genotypes with desirable characters. (Bonea and Urechean, 2015). The chromosome morphology of *A. cepa* is very easily changed by chemicals. The *Allium* assay proved to be a suitable

model system for measuring the cytogenotoxic potential of different chemicals. Therefore, chromosome damage has become a relevant testing method (Butnaru et al., 2004; Sărac, 2005; Baciu et al., 2009, Bonciu, Rosculete et al., 2018; Rosculete et al., 2019).

The results obtained indicated that Tz induced a strong clastogenic and genotoxic effect in meristematic cells to *A. cepa*, by reduction of the mitotic index and occurrence a different cytological abnormalities.

Table 1

Cytogenetic effects of Tartrazine to *A. cepa*

Variants/ Conc. (%)	MI ± SE %	Cells abnormalities frequency (%)				TA (%)
		L	S	CM	MN	
V1 (Control)	28.12±0.50	0	0.01	1.52	0	1.53
V2/0.3	20.22±0.35	5.12	3.10	3.25	2.18	13.65
V3/1.0	11.28±0.40*	8.15	6.01	7.10	3.20	24.46
V4/1.3	7.55±0.55*	9.36	6.85	10.26	3.62	30.09

MI = Mitotic index; SE = Standard error; L = Laggards; S = Stickiness; CM = C-mitosis; MN = Micronucleus; TA = Total abnormalities; *Significant at level 5% (p=0.05)

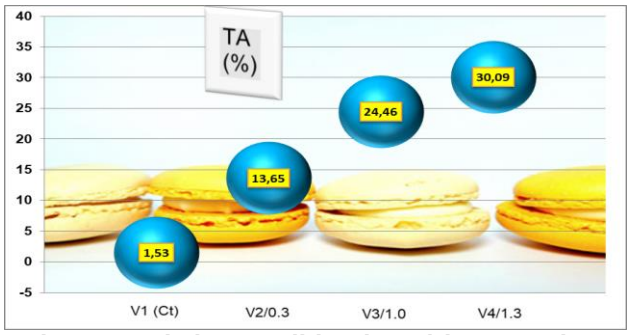

Fig. 2. Total abnormalities (TA%) increase in meristematic tissues of A. cepa exposed to tartrazine

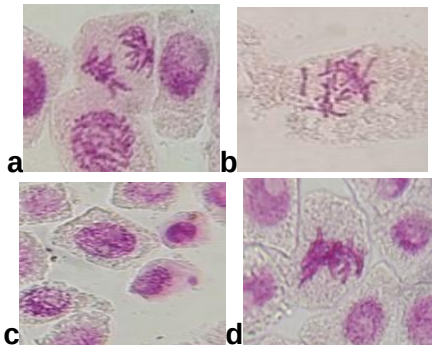

Fig. 3. Cytogenetic abnormalities identified in meristematic tissues of A. cepa exposed to tartrazine: laggards in anaphase (a); C-mitosis (b); micronucleus (c) and sticky metaphase (d)

CONCLUSIONS

This study highlights a strong cytotoxic and clastogenic effect induced by Tz in onion cells manifested by inhibited of the mitotic activity, as well as by the occurrence of several types of chromosome aberrations in mitosis and nuclear abnormalities in interphase. These results suggest prudence regarding the consumption of foods which containing tartrazine.

BIBLIOGRAPHY

1. **Baciu, A., Sărac, I., Mike, L.**, 2009 - *Genetica si ameliorarea cartofului (Solanum tuberosum L.)*, Ed. Eurobit, Timisoara.

2. **Bonciu, E.,** 2012 - *Cytological effects induced by Agil herbicide to onion,* Journal of Horticulture, Forestry and Biotechnology, 16(1): 68-72.

3. **Bonciu, E**., 2017 - *Food processing, a necessity for the modern world in the context of food safety: A review*, Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series, 47(1): 391-398.

4. **Bonciu, E., Roşculete, E., Olaru, A.L., Roşculete, C.A.**, 2018 - *Evaluation of the mitodepresive effect, chromosomal aberrations and nuclear abnormalities induced by urea fertilization in the meristematic tissues of Allium cepa L.*, Caryologia , International Journal of Cytology, Cytosystematics and Cytogenetics, 71(4), 350-356.

5. **Bonciu, E., Firbas, P., Fontanetti, C.S., Wusheng, J., Karaismailoğlu, M.C., Liu, D., Menicucci, F., Pesnya, D.S., Popescu, A., Romanovsky, A.V., Schiff, S., Ślusarczyk, J., De Souza, C.P., Srivastava, A., Sutan, A., Papini, A.**, 2018 - *An evaluation for the standardization of the Allium cepa test as cytotoxicity and genotoxicity assay*, Caryologia, 71(3), 191-209.

6. **Bonciu, E**., 2018 - *Evaluation of cytotoxicity of the herbicide Galigan 240 EC to plants*, Agricultural Sciences &

Veterinary Medicine University, Bucharest. Scientific Papers. Series A. Agronomy, Vol. LXI(1), 175-178.
7. **Bonea, D., Urechean, V.,** 2015 – *The evaluation of heterosis for Romanian maize germplasm*, Pakistan Journal of Botany, vol. 47(6): 2387-2390.
8. **Bonea, D.,** 2016 - *Effect of the aqueous extracts of Amoracia rusticana L. on the seed germination and seedling growth of Zea mays L. under drought stress,* Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series. Vol.46 No.1 pp.56-61.
9. **Bonea, D.,** 2016 -*The effect of climatic conditions on the yield and quality of maize in the central part of Oltenia*, Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series. Vol.46 No.1 pp.48-55.
10. **Bostan, C., Butnariu, M., Butu, M., Ortan, A., Butu, A., Rodino, S., Parvu, C**., 2013 - *Allelopathic effect of Festuca rubra on perennial grasses*, Romanian biotechnological letters, 18(2), 8190–8196.
11. **Bozhanska, T.**, 2018 - *Botanical and morphological composition of artificial grassland of bird's–foot–trefoil (Lotus Corniculatus L.) treated with lumbrical and lumbrex*, Banat's Journal of Biotechnology, IX(19), 12-19.
12. **Butnariu, M**., 2012 - *An analysis of Sorghum halepense's behavior in presence of tropane alkaloids from Datura stramonium extract*s, Chemistry Central Journal, 6, no 75.
13. **Butnaru, G., Căpâlnăşan, I., Sărac, I., Jurca, M., Baciu, A., Popescu, C., Avramescu, A., 2004 -** *Cromosomii – particularităţi morfo-funcţionale la plante şi animale*, Editura Mirton, Timișoara. ISBN 973-661-288-0, 145 p.
14. **Georgieva, N., Nikolova, I., Naydenova, Y.**, 2018 - *Possibility for weed control by using of an organic product with herbicidal effect*, Banat's Journal of Biotechnology, IX(17), 40-49.
15. **Ianculov, I., Palicica, R., Butnariu, M., Dumbrava, D., Gergen, I**., 2005 - *Achieving the crystalline state of chlorophyll of the Fir–tree (Abies alba) and the pine* (*Pinus sylvestris),* Revista de chimie, 56(4), 441–443.
16. **Righi, K., Assia Righi, F., Boubkeur, A., Boungab, K., Elouissi, A., Djendara, A.C.**, 2018 - *Toxicity and repellency of three Algerian medicinal plants against pests of stored product: Ryzopertha dominica (Fabricius) (Coleoptera: Bostrichidae),* Banat's Journal of Biotechnology, IX(17), 50-59.
17. **Rosculete, C.A., Bonciu, E.; Rosculete, E., Olaru, L.A**., 2019 - *Determination of the Environmental Pollution Potential of Some Herbicides by the Assessment of Cytotoxic and Genotoxic Effects on Allium cepa*, Int. J. Environ. Res. Public Health, 16(1), 75.
18. **Samfira, I., Butnariu, M., Rodino, S., Butu, M**., 2013 - *Structural investigation of mistletoe plants from various hosts exhibiting diverse lignin phenotypes*, Digest journal of nanomaterials and biostructures, 8(4), 1679–1686.
19. **Sărac, I.**, 2005 - *Genetica si ameliorarea speciilor forestiere*, Ed. Mirton, Timisoara.
20. **Vazhangat, P., Thoppil, J.E**., 2016 - *Apoptotic induction via membrane/DNA damage and metabolic inactivation by synthetic food colorants in Allium cepa root meristem*, Turk. J. Biol., 40, 922-933.
***FDA, 2016 - Overview of food ingredients, additives & colors. Av. on http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm094211.htm
***https://www.safefood.eu/Food-Colour-Resource/Food-Colour/E102.asp